Anaerobic co-digestion of cheese whey and the screened liquid fraction of dairy manure in a single continuously stirred tank reactor process: Limits in co-substrate ratios and organic loading rate

Carlos Rico[a,*], Noelia Muñoz[b,c], José Luis Rico[c]

[a]Department of Water and Environmental Science and Technologies, University of Cantabria, Avda. Los Castros, s/n, 39005, Santander, Spain

[b]Energy Lab (Sustainable Energy Technological Center), Edificio CITEXVI, Local 1 R/ Fonte das Abelleiras, s/n, Campus Universitario de Vigo 36310, Vigo, Spain.

[c]Department of Chemical and Process Engineering Resources, University of Cantabria, Avda. Los Castros, s/n, 39005, Santander, Spain.

* Corresponding author. Tel.: +34 942201848; fax: +34 942201703.
E-mail address: ricoc@unican.es (Carlos Rico)

Abstract

Mesophilic anaerobic co-digestion of cheese whey and the screened liquid fraction of dairy manure was investigated with the aim of determining the treatment limits in terms of the cheese whey fraction in feed and the organic loading rate. The results of a continuous stirred tank reactor that was operated with a hydraulic retention time of 15.6 days showed that the co-digestion process was possible with a cheese whey fraction as high as 85% in the feed. The efficiency of the process was similar within the range of the 15-85% cheese whey fraction. To study the effect of the increasing loading rate, the HRT was progressively shortened with the 65% cheese whey fraction in the feed. The reactor efficiency dropped as the HRT decreased but enabled a stable operation over 8.7 days of HRT. At these operating conditions, a volumetric methane production rate of 1.37 m^3 CH_4 m^{-3} d^{-1} was achieved.

Keywords

Biogas; Co-digestion; CSTR; Cheese Whey; Organic Loading Rate.

1. Introduction

Cheese whey is the liquid remaining after the precipitation and removal of milk casein during cheese-making (Teixeira et al., 2010). From a valorization point of view, cheese whey is a nutrient-rich by-product that contains approximately 55% of the initial milk nutrients (Prazeres et al., 2012). Among the most abundant of these nutrients are lactose (4.5–5% w/v), soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v) and mineral salts (8–10% of the dried extract) (Guimarães et al., 2010). Despite its nutritional value, large volumes of cheese whey are discharged into the environment every day (Saddoud et al., 2007). The problem occurs due to the high water content of cheese whey (92-95%), which increases the cost of transportation. In addition, the use of valorization technologies can make the use of cheese whey uneconomical, in particular for small- and medium-sized cheese producing units. When the costs associated with valorization technologies are not reasonable, the disposal of cheese whey becomes an environmental problem.

Cheese whey has a high organic load (up to 80 g COD L^{-1}) and a high biodegradability (Kalyuzhnyi et al, 1997; Mawson, 1994) that causes excess oxygen consumption if it is directly disposed of in water bodies. Given these characteristics, biological treatment processes are required when cheese whey needs to be managed as a waste effluent. Due to the high organic content of whey, aerobic treatment processes such as the activated sludge process are completely inappropriate (Gavala et al., 1999). Thus, anaerobic treatment is a particularly attractive solution for treating or pre-treating this waste effluent because it offers an excellent solution in terms of both energy savings and pollution control (Ferreira et al., 2014; Ergüder et al., 2001).

However, some difficulties have been reported regarding the anaerobic digestion of cheese whey. The majority of these difficulties are due to the low alkalinity content and the rapid acidification of cheese whey that can exhaust the buffering capacity, leading to a drop in pH, volatile fatty acids (VFA) accumulation and subsequent reactor failure (Ergüder et al., 2001; Kalyuzhnyi et al, 1997). Another drawback for the anaerobic treatment of cheese whey is the difficulty in obtaining granulation and the tendency to produce an excess of viscous exopolymeric materials that severely reduces sludge settleability and can cause biomass washout in high-load anaerobic reactors (Malaspina et al., 1995).

To address the above difficulties, the co-digestion of cheese whey with animal manure in a CSTR digester has proven to be a solution because manure can provide the necessary buffer capacity to ensure the stability of the process (Gelegenis et al, 2007; Kavacik and Topaloglu, 2010). In addition, co-digestion of animal manure with different substrates may enhance the anaerobic digestion process due to a better carbon and nutrient balance, which results in a suitable option for improving biogas production (El-Mashad et al., 2010; Risberg et al., 2013). It has been recognized that using animal manure alone, although convenient and feasible, may not represent the most efficient way to produce biogas due to its inherently low C/N ratio (Wu et al., 2010). The optimal C/N ratio for bacterial growth in anaerobic digestion systems has been reported to range from 20-30, although the optimal C/N ratio varies with the type of digested feedstock (Yan et al., 2015). In this regard, the characteristics of dairy manure and cheese whey are completely opposite. Dairy manure presents many suspended solids and fibrous material and only a small part of the organic matter content is in the soluble form. Dairy manure has enough alkalinity to develop the anaerobic process and its anaerobic

biodegradability is approximately 45% (Rico et al., 2007). Hydrolysis is the rate-limiting step in the anaerobic digestion of dairy manure, whereas for cheese whey it is methanogenesis. The higher C/N ratio of cheese whey can also provide a more optimal C/N ratio and thus can have a positive synergetic effect on gas production. Dairy manure and cheese whey are totally antagonistic but complementary for the anaerobic co-digestion process. Indeed, anaerobic co-digestion of animal manure and by-products from the food industry demonstrates many advantages. Biogas plants can convert a disposal problem into a profit centre, allowing animal manures and food wastes to be converted into highly valuable fuel, reducing greenhouse gases emissions and replacing mineral fertilization with nutrient recovery (Agyeman and Tao, 2014; Holm-Nielsen et al., 2009).

To date, very few attempts have been made for the co-digestion of dairy manure and cheese whey in a continuous-mode operation (Bertin et al., 2013; Comino et al., 2012; Kavacik and Topaloglu, 2010; Lo et al., 1988). In the present work, the anaerobic co-digestion of cheese whey and the screened liquid fraction of dairy manure has been investigated in a single continuously stirred tank reactor (CSTR) process at 35ºC. More specifically, the aim was to reach the treatment limits in terms of: a) cheese whey fraction in the feed under a constant hydraulic retention time (HRT) and b) organic loading rate (OLR) by decreasing the HRT operating with a constant cheese whey fraction in the feed.

2. Materials and methods

2.1. Substrates

Cheese whey (CW) was supplied by Queserías la Fuente, a dairy milk processor located in Heras (Cantabria, Spain). The CW was transported to the laboratory and stored at 4ºC prior to use. The CW characteristics (Table 1) were quite uniform during the experimentation process as indicated by the relative low SD in the CW characteristics.

The screened liquid fraction (SLF) of dairy manure used as a co-substrate was obtained from a pilot plant located in the ''La Granja'' agricultural secondary school (Heras, Cantabria). Dairy manure was collected from the manure pit of a 500-free stall dairy cow farm equipped with scrape systems. The manure was extracted from the dung pit by a tractor equipped with a vacuum tank system and transported to the pilot plant.

The raw manure was separated by means of a screw press separator (Doda MS5CE, 0.8 mm mesh). The SLF was processed and collected from the pilot plant, delivered to lab installations and stored at 4ºC prior to use. The SLF characteristics were not uniform due to the manure pit management and weather conditions but were reasonably consistent during the experimental period to ensure the reliability of the experiments. The mean characteristics of both substrates during experimentation are shown in Table 1.

2.2. Experimental setup scheme

The experimental setup scheme of the developed process is shown in Fig. 1. The manure separation processes to obtain SLF were conducted at the pilot scale. More details about the pilot installation and the separation process can be found in Rico et al. (2011). The CSTR was operated at the lab scale.

2.3. CSTR digester

The CSTR digester was cylindrical (vertical type), made of PVC and 36 cm in internal diameter and 24 cm high with an operating volume of 21 L. A vertical mechanical stirrer (40 rpm) was used for mixing and homogenization of the digester content and to avoid stratifications inside the reactor. It was programmed to work fifteen minutes per hour. The digester was fed manually once a day. The anaerobic effluent left the reactor when the digester was fed by means of an exit tube with a hydraulic closing system to prevent the entrance of air.

The CSTR was equipped with a temperature probe. A stable reactor temperature was maintained at 35ºC by means of the continuous flow of heated water through a helical coiled tube heat exchanger inside the reactor.

Biogas was released from the reactor by its own pressure through a tube in the dome. The volume of biogas generated in the CSTR was measured by means of a home-made biogas meter device constructed using two coaxial chambers made of acrylic cylinders which were interconnected by means of two small holes in the lower zone of the internal chamber. The internal chamber was closed on the top and arranged with a piping connection to a three-way solenoid valve with biogas from the CSTR inlet and exhaust. A pre-set magnetic level sensor regulated the operation of the three-way valve to release the collected biogas, resetting the entire system. The total volume of biogas is

the product of multiplying the number of cycles (fillings or emptyings) which were recorded by a counter system by the volume of the chamber. After passing through the gas meter device, the biogas was stored for a day in a biogas holder where four samples were collected to determine the daily average biogas composition.

2.4. Mode of operation

Previous to the co-digestion operation the CSTR digester had been operating with SLF alone. For this reason, the process start-up was easy to perform because the CSTR was filled with stabilized anaerobic biomass.

The CSTR was fed in a semi-continuous mode of operation. The HRT was calculated by dividing the operating volume of the CSTR by the daily volume of the fed substrate mixture. The duration of the CSTR experiment was 240 days, and the experiment was divided into two stages.

The feed ratios or HRT were changed after 20 days of operation at the current loading rate. For each operating condition the first 11 days were used to allow the digester to reach stationary conditions. From days 12 to 20, influent and effluent samples were collected every other day (five samples) and their characteristics were determined (duplicate analyses). The parameters analysed were pH, alkalinity, TS, VS, COD and VFA. The biogas production and methane content in biogas were determined daily. The influent, effluent and biogas samples were analysed immediately after sampling.

7

Based on previous unpublished experiences, during the first experimentation stage, the HRT of the CSTR reactor was set to 15.6 days. Starting from a substrate mixture ratio of 15:85 (v/v CW:SLF), the CW fraction was progressively increased by 10% up to 85%. Operation at a constant HRT lasted 160 days. After this period a second experimentation stage was performed in which the CW fraction in the feed was set at 65%, and the HRT was progressively reduced starting from 13.1 days to check the limits in terms of the HRT and OLR.

2.5. Biochemical methane potential and residual methane yield tests

Biochemical methane potential (BMP) tests for CW and SLF alone and residual methane yield (RMY) tests for the anaerobic effluents (digestates) were conducted in duplicate in 500 mL serum bottles capped with rubber septum sleeve stoppers, which were used as reactors.

The BMP of CW and SLF were determined during the experimental period. During the experimental period, four samples of CW and SLF were tested to determine their methane potential. For the BMP determination of CW and SLF each bottle was filled with 300 g of the substrate and inoculum with a $VS_{inoculum}/VS_{substrate}$ ratio of 1. An anaerobically digested liquid fraction of dairy manure (22.6 g TS L^{-1}; 13.5 g VS L^{-1}) was used as the inoculum. Previous to the BMP tests, the inoculum was degassed for five days at 35ºC. The results are expressed as the means ± SD and by subtracting methane production from the blanks (inoculum). For the RMY determination each reactor was fed with 400 g of digestate.

After set-up of the reactors helium was used to flush out the air in the headspace of the bottles and thereafter incubated at 35ºC for 30 days. All of the reactors were manually stirred once a day. The gas production was determined by pressure measurement. The pressure was measured from the headspace of the reactors through the septum with a syringe connected to a digital pressure sensor with silicon measuring cell (ifm, type PN78, up to 2 bar). Biogas samples were also collected through the septum by a needle connected to a syringe.

2.6. Analytical techniques

VFAs were determined using a HP6890 gas chromatograph (GC) fitted with a 2 m 1/8-in glass column, liquid phase 10% AT 1000, packed with solid-support Chromosorb W-AW 80/100 mesh. Nitrogen was used as the carrier gas at a flow rate of 14 mL/min, and a FID detector was installed. The total VFA concentration is expressed in COD units (COD_{VFA}). The biogas composition was assayed on a 2 m Poropak T column in a HP 6890 GC system with helium as the carrier gas at a flow rate of 15 mL/min with a TCD detector. The biogas and methane volumes are expressed at 0ºC and 1 atm in dry conditions. The influent and effluent pHs were measured from samples with a glass electrode pH meter (WTW, SENTIX 21). The bicarbonate alkalinity (BA) and the volatile acids alkalinity (VA) were determined by titration at a pH of 5.1 and 3.5, respectively, according to the method described by Anderson and Yang (1992). All of the other analyses were performed according to the Standard Methods (APHA, 1998).

3. Results and discussion

3.1. Composition of substrates

The composition and BMP of CW and SLF are presented in Table 1. These data reveal that CW had a slighter higher organic content (57.5 ± 1.8 g COD L^{-1}) compared with SLF (53.2 ± 4.5 g COD L^{-1}), but the composition of CW was more constant than that of the SLF, as shown by their standard deviation. Due to the higher methane potential yield of CW, higher volumetric methane production rates should be achieved with a higher CW proportion in the feed mixture. On the contrary, the SLF contributes to the buffering capacity due to the high levels of BA (159 ± 67 meq L^{-1}). The C/N ratio of CW was 22.1, which was higher than that of the SLF (9.1) and contributes to a better C/N ratio in the feed mixture as the CW fraction increases.

Table 1 goes here

3.2. Operation at a constant HRT (15.6 days)

The characteristics of the CSTR influents and effluents during this stage are provided in Table 2. The CSTR was operated with increasing CW in the feed. Starting from a 15% CW fraction in the fed, it was increased by 10% up to 85% CW. The VS content in the feed increased from 31.2 g L^{-1} for the feed mixture consisting of 15% CW to 46.4 g L^{-1} for the 85% CW feed. As a result of this increase the organic loading rate (OLR), in terms of the VS applied to the CSTR, also increased, showing a significant correlation ($p<0.05$) with the CW fraction. The applied OLR was augmented with increasing CW in the feed (from 2.0 kg VS m^{-3} d^{-1} (15% CW) to 3.0 kg VS m^{-3} d^{-1} (85% CW)). For the VFA, a negative Pearson correlation was observed between the CW fraction and the VFA content in the feed mixture ($p<0.05$). However the pH and the alkalinity (BA and VA) did not show significant correlations with the CW content in the mixture. Based on

the C/N ratios of CW and SLF, the C/N ratio of the mixture varied in the range of 11.1 to 20.2 for 15% CW and 85% CW, respectively.

Table 2 goes here

The effluent characteristics are located in Table 2. The organic removal rate (ORR) and the volumetric methane production rates in Fig. 2 show a stable and efficient CSTR reactor operation with a minimal presence of VFA in the effluents for all of the experimental conditions. The effluent pH decreased with an increasing CW fraction in the feed but remained in a range between 7.9 (15% CW) and 7.1 (85% CW). With regards to the alkalinity in the effluent, the ratio VA/(BA+VA) increased with an increasing CW fraction in the feed but was lower than 0.3 for all of the experimental conditions, which is the typical recommended value needed to guarantee stability in anaerobic digestion processes (Martín-González et al., 2013). The highest value was found for the operation with 85% CW in the feed, resulting in a ratio of VA/(BA+VA) in the effluent 0.12, which made the process stable without the risk of acidification.

In Fig. 2a, the organic loading rate (OLR) and the organic removal rate (ORR) are presented against the CW fraction in the feed. The process achieved a VS removal percentage between 56.2% for the CW fraction of 15% and 69.9% for the CW fraction of 85%. Due to the higher biodegradability of CW, VS removal increased with the increasing CW fraction in the feed mixture. Indeed, the ORR exhibited a good Pearson correlation (p<0.01) with the CW fraction in the feed.

11

Fig. 2 goes here

The volumetric methane production rate also exhibited a good Pearson correlation ($p<0.05$) with OLR (Fig. 2b). The CSTR yielded 0.53 ± 0.03 m^3 CH$_4$ m^{-3} d^{-1} when the CW fraction in the feed was 15%. This rate increased progressively when the CW:SLF ratio in the feed was increased, reaching its highest value for 85% CW (0.91 ± 0.04 m^3 CH$_4$ m^{-3} d^{-1}). The methane content in the biogas diminished with increasing CW content in the feed (Fig. 2b). In this case, a negative Pearson correlation was observed between the methane content in the biogas and the OLR ($p<0.05$). Under the first condition of the 15% CW fraction in the feed, the methane content in the biogas was 68.7 ± 1.2%. For the last condition of 85% CW in the feed, the methane content in the biogas was 57.6 ± 1.1%. This result can be attributed to the difference in organic compounds between the CW and the SLF. As shown in Table 1, VFA is present in the SLF but not in the CW. The organic compounds in the CW must be processed by various groups of microorganisms; consequently, higher CW:SLF ratios in the feed resulted in a higher CO$_2$ content in the biogas produced.

3.3. Operation at a constant CW:SLF ratio (65:35) and decreasing HRT

After operation with different CW:SLF ratios at a constant HRT of 15.6 days, the CSTR was fed with a mixture consisting of 65% CW and 35% SLF to check the process limit in terms of HRT for a period of 80 days. The operation started with an initial HRT of 13.1 days. The HRT was gradually decreased until symptoms of efficiency decay were observed. The HRTs applied were 13.1, 11.3, 10.0 and 8.7 days. In this case, as the CW fraction in the feed was constant, decreases in the HRT were accompanied by increases

in the OLR due to the lower HRT. The characteristics of the CSTR influents and

effluents during this stage are provided in Table 3 (data corresponding to 15.6 days of

HRT are those from stage 1). The OLR applied during this experimental period varied

from 2.7 kg VS m^{-3} d^{-1} for 15.6 days of HRT to 5.9 kg VS m^{-3} d^{-1} for 8.7 days of HRT,

which is a 97% higher OLR than the maximum OLR applied during stage 1.

Table 3 goes here

The data in Table 3 and Fig. 3 show the good performance of the system. As expected

for a continuous anaerobic digestion process, the VS removal percentage decreased with

decreasing HRT, from 67.2% for 15.6 days of HRT to 59.1% for 8.7 days of HRT. The

ORR exhibited a good Pearson correlation ($p<0.01$) with the applied OLR and between

the OLR and the volumetric methane production rate. In Fig. 3b, the volumetric

methane production rate and the methane content in biogas for this period are shown.

The CSTR yielded 0.85 m^3 CH_4 m^{-3} d^{-1} at 15.6 days of HRT. This rate increased

progressively with decreasing HRT, reaching its highest value at 8.7 days of HRT (1.37

m^3 CH_4 m^{-3} d^{-1}). The methane content in the biogas decreased as the OLR increased.

The highest value was found at 15.6 days of HRT (58.4 % CH_4), and the lowest value

was found at 8.7 days of HRT (53.0% CH_4). In this case, the decrease in methane

content in the biogas was caused by the decreasing HRT and increasing OLR.

The VFAs in the effluent remained at negligible values for 15.6 and 13.1 days of HRT

but increased their concentration from 11.3 days of HRT when 0.70 g COD_{VFA} L^{-1} was

detected. At 10 days of HRT, the VFA in the effluent reached 0.77 g COD_{VFA} L^{-1}.

During operation at an HRT of 8.7 days, the effluent COD_{VFA} increased and reached a

mean value of 2.6 g COD_{VFA} L^{-1} at this operating condition. The presence of VFA in the effluent at such concentrations indicated that the capacity of the bacteria present in the reactor to process the incoming substrate had been overcome. This HRT can be considered the process performance limit under these conditions. Although the reactor was performing at a stable operation condition ((VA/(BA+VA) = 0.2) and the effluent pH was in a secure value (7.1), the reactor was stopped because a lower HRT could cause the process to become unstable.

Fig. 3 goes here

3.4. Residual methane yields

The residual methane yield of the digestates in the study was in the range of 1.0 to 3.8 L CH_4 kg^{-1} of digestate (73 – 182 L CH_4 kg^{-1} VS). The residual methane yields along with the efficiency of the process for the different operating conditions are shown in Fig. 4. The efficiency of the process represents the percentage of the methane potential of the feed mixture that was produced in the CSTR process and has been calculated according to the following equation:

$$Efficiency\ (\%) =\ 100 \cdot \frac{Vol_{CH_4} \cdot HRT}{Vol_{CH_4} \cdot HRT + RMY}$$

Vol_{CH4} = Volumetric methane production rate (L CH_4 $L_{reactor}^{-1}$ d^{-1})

HRT = Hydraulic retention time (d)

RMY = Residual methane yield (L CH_4 $L_{digestate}^{-1}$)

As can be observed in Fig. 4a, the residual methane yields and the efficiency obtained under operation at 15.6 days of HRT were quite similar. The residual methane yields

ranged between 1.0-1.4 L CH_4 kg^{-1} digestate, whereas the efficiency ranged between 89.3% and 91.8%. In this regard, increased C/N ratios with higher CW fractions in the feed mixture did not improve the efficiency of the process. This can be explained by the fact that low C/N ratios increases the risk of ammonia inhibition, but NH_4^+-N levels in SLF were below inhibition limits reported by Chen et al. (2008). On the contrary, in Fig. 4b it can be observed that the residual methane yields for the digestate samples obtained during the operation at different HRT with a 65% CW fraction of the feed increased with decreasing HRT. The higher residual methane yields entailed a decrease in the efficiency of the process. The residual methane yield increased from 1.3 L CH_4 kg^{-1} of digestate for 15.7 days of HRT to 3.8 L CH_4 kg^{-1} for 8.7 days of HRT, which represented a drop in the efficiency from 90.8% to 75.9%. During the operation with the same influent feed, when the HRT diminishes the OLR increases, which would reduce the percentage of organic matter removal and reactor performance if the methanogenic activity of the biomass involved in the process was the same. In our process the increasing VFA concentration found in the effluent during operation as a result of shortening the HRT also explains the drop in the efficiency of the process.

Fig. 4 goes here

3.5. Comparison with CSTR anaerobic co-digestion processes for cheese whey and animal manure

Even though there are many reports in the literature regarding the anaerobic co-digestion of cheese whey with manure alone and combined with other substrates, many of these studies have been performed under batch conditions, which are not useful to predict the continuous operation in CSTR systems. Very few studies have attempted to

address the anaerobic co-digestion of cheese whey and animal manure in continuous-mode operation. A comparison between the results from the present work with other previous works dealing with the anaerobic co-digestion of cheese whey and animal manure in CSTR systems is provided in Table 4. All of these authors reported that the alkalinity provided for manure ensured the stability of the process within the CW fractions shown in Table 4. Gelegenis et al. (2007) proved that anaerobic co-digestion of CW and diluted poultry manure was possible without chemical addition but reported unstable process performance when the CW fraction was over 50% in the feed mixture. Kavacik and Topaloglu (2010) studied the anaerobic co-digestion of CW with diluted and screened dairy manure. Based on the results from Gelegenis et al. (2007), these authors did not test the process with CW fractions higher than 50% but reported that it was necessary to start the co-digestion process with a mixture of CW and digested manure. Comino et al. (2012) reported that the anaerobic co-digestion of CW and dairy manure was possible with a CW fraction up to 65% CW, but the best results were obtained with a 50% CW fraction in the feed. Bertin et al. (2013) performed a two-stage anaerobic co-digestion process with a 50% CW fraction in the feed. These authors did not try the continuous operation process with higher CW fractions on the basis of the results obtained by batch tests where they observed acidification with CW fractions higher than 60%.

Table 4 goes here

4. Conclusions

The results from this study have demonstrated that the difficulties in the anaerobic

16

digestion of cheese whey, such as acidification, can be solved by co-digesting cheese whey with a small proportion of liquid dairy manure. Although a higher cheese whey proportion enabled higher methane yields, likely due to the its higher biodegradability, similar process efficiencies were also observed for mixtures within the range of a 15-85% cheese whey fraction operating at an HRT of 15.6 days. The reactor efficiency dropped when the HRT decreased, but a stable process operation was reached at a HRT as short as 8.7 days.

References

1. Agyeman, F.O., Tao, W., 2014. Anaerobic co-digestion of food waste and dairy manure: Effects of food waste particle size and organic loading rate. J. Environ. Manag. 133, 268-274.

2. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

3. Anderson, G.K., Yang, G., 1992. Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration, Water Environ. Res. 64, 53–59.

4. Bertin, L., Grilli, S., Spagni, A., Fava, F., 2013. Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. Bioresour. Technol. 128, 779-783.

5. Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044-4064.

6. Comino, E., Riggio, V., Rosso, M., 2012. Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. Bioresour. Technol. 114, 46-53.

7. El-Mashad, H.M., Zhang, R., 2010. Biogas production from co-digestion of dairy manure and food waste. Bioresour. Technol. 101, 4021-4028.

8. Ergüder, T., Tezel, U., Güven, E., Demirer, G.N., 2001. Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors. Waste Manag. 21, 643-650.

9. Ferreira, P.A., Santos, S.C., Kimiko, I., Amâncio, M.B., Luiz Silva, E., 2014. Hydrogen production from cheese whey with ethanol-type fermentation: Effect of hydraulic retention time on the microbial community composition. Bioresour. Technol. 161, 10-19.

10. Gavala, H.N., Skiadas, I.V., Lyberatos, G., 1999. On the performance of a centralised digestion facility receiving seasonal agroindustrial wastewaters. Water Sci. Technol. 40, 339–346.

11. Gelegenis, J., Georgakakis, D., Angelidak, I., Marris, V., 2007. Optimization of biogas production by co-digesting whey with diluted poultry manure. Renew. Energ. 32, 2147-2160.

12. Guimarães, P., Teixeira, J.A., Domingues, L., 2010. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol. Adv. 28, 375–384.

13. Kalyuzhnyi, S.V., Pérez, E., Rodriguez, J., 1997. Anaerobic treatment of high-strength cheese-whey wastewaters in laboratory and pilot UASB-reactors, Bioresour. Technol. 60, 59-65.

14. Kavacik, B., Topaloglu, B., 2010. Biogas production from co-digestion of a mixture of cheese whey and dairy manure. Biomass Bioenerg. 34, 1321-1329.

15. Lo, K.V., Liao, P.H., Chiu, C., 1988. Mesophilic anaerobic digestion of a mixture of cheese whey and dairy manure. Biomass 15, 45-53.

16. Malaspina, F., Stante, L., Cellamare, C.M., Tilche, A., 1995. Cheese whey and cheese factory wastewater treatment with a biological anaerobic-aerobic process. Water Sci. Technol. 32, 59-72.

17. Martín-González, L., Font, X., Vicent, T., 2013. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochem. Eng. J. 76, 1-5.

18. Mawson, A.J., 1994. Bioconversions for whey utilization and waste abatement. Bioresour. Technol. 47, 195-203.

19. Prazeres, A.R., Carvalho, F., Rivas, J., 2012. Cheese whey management: A review. J. Environ. Manag. 110, 48-68.

20. Rico, J.L., García, H., Rico, C., Tejero, I., 2007. Characterisation of solid and liquid fractions of dairy manure with regard to their component distribution and methane production, Bioresour. Technol. 98, 971–979.

21. Rico, C., Rico, J.L., Tejero, I., Muñoz, N., Gómez, B., 2011. Anaerobic digestion of the liquid fraction of dairy manure in pilot plant for biogas production: residual methane yield of digestate, Waste Manag. 31, 2167–2173.

22. Risberg, K., Sun, L., Levén, L., Horn, S.J., Schnürer, A., 2013. Biogas production from wheat straw and manure – Impact of pretreatment and process operating parameters. Bioresour. Technol. 149, 232–237.

23. Saddoud, A., Hassaïri, I., Sayadi, S., 2007. Anaerobic membrane reactor with phase separation for the treatment of cheese whey.

24. Teixeira, K., Pereira M.A., Nicolau, A., Dragone, G., Domingues L., Teixeira J.A., Almeida e Silva, J.B., Schwan, R.F., 2010. Production of fermented cheese whey-based beverage using kefir grains as starter culture: Evaluation of morphological and microbial variations. Bioresour. Technol. 101, 8843–8850.

25. Wu, X., Yao, W., Zhu, J., Miller, C., 2010. Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresour. Technol. 101, 4042-4047.

26. Yan, Z., Song, Z., Li, D., Yuan, Y., Liu, X., Zheng, T., 2015. The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. Bioresour. Technol. 177, 266-273.

Figure captions

Figure 1. Experimental set up scheme.

Figure 2. Performance data of the CSTR at a constant HRT (15.6 days) and increasing the CW content in the feed: (a) Organic Loading Rate and Organic Removal Rate; (b) Volumetric methane production rate and methane content in the biogas. Mean values ± SD.

Figure 3. Performance data of the CSTR at a constant CW fraction in the feed (65%) and a decreasing HRT: (a) Organic Loading Rate and Organic Removal Rate; (b) Volumetric methane production rate and methane content in the biogas. Mean values ± SD.

Figure 4. Residual methane yields and efficiency of the process: a) during stage 1 – constant HRT (15.6 days); b) during stage 2 – constant CW fraction in the feed (65%).